Lab Ultrafiltration

Tips and Tricks

Simplifying Progress

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Build Knowledge Though Experiments

The use of ultrafiltration membranes for concentration and purification of proteins and DNA is ubiquitous in biological laboratories. Filter devices with ultrafiltration membranes can also be used for concentration of other macromolecules such as inorganic polymers, nanoparticles or even viruses. Although performing sample concentration and buffer exchange using an ultrafiltration device is relatively simple, some tricks of the trade can improve your recovery or speed up your work flow considerably.

The following Application Notes will give you an overview of how to:

Desault Samples	Concentrate Samples	Recovery
Desalting and Buffer Exchange with Vivaspin® Centrifugal Concentrators	Concentration to a defined final volume with Vivaspin® Turbo 15, Vivaspin® Turbo 4 and Vivaspin® 500	Treatment of Vivaspin® concentrators for improved recovery of low-concentrated protein samples

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Application Note

April 03, 2019

Keywords or phrases:
Diafiltration, buffer, exchange, de-salting

Desalting and Buffer Exchange With Vivaspin® Centrifugal Concentrators

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Abstract

This short application note highlights the ability to reduce protein sample salt concentrations by up to 99%, or to exchange the buffer sample entirely, using Vivaspin® 20 and Vivaspin® 6 centrifugal ultrafiltration devices. This process is known as ‘diafiltration’ and prevents the over concentration of proteins with a tendancy to precipitate at higher salt concentration.

Introduction

Vivaspin® centrifugal concentrators, with patented vertical membrane technology, combine fast filtration with high recovery of target proteins. This makes Vivaspin® the technology of choice for desalting or buffer exchange, avoiding lengthy dialysis steps.

While proteins are retained by an appropriate ultrafiltration membrane, salts can pass freely through, independent of protein concentration or membrane MWCO. In consequence, the composition of the buffer in the flow-through and retentate is unchanged after protein concentration. By diluting the concentrate back to the original volume, the salt concentration is lowered. The concentrate can be diluted with water or salt-free buffer if simple desalting is required; however, it is also possible to dilute the concentrate with a new buffer, thereby exchanging the buffering substance entirely. For example, a 10 ml protein sample containing 500 mM salt, if concentrated 100× still contains 500 mM salt. If this concentrate is then diluted 100× with water or saltfree buffer, the protein concentration returns to normal, while the salt concentration is reduced 100× to only 5 mM, (I.E. a 99% reduction in salt).

The protein sample can then be concentrated again to the desired level, or the buffer exchange can be repeated to reduce the salt concentration even further before a final concentration of the protein. This process is called "diafiltration". For proteins with a tendency to precipitate at higher concentrations, it is possible to perform several diafiltration steps in sequence, with the protein concentrated each time to only 5 or 10x. For example, if a precipitous protein sample is concentrated to 5x then diluted back to the original volume, and this process is repeated a further two times, this still results in a >99% reduction in salt concentration, without over concentrating the protein.

Desalting and Buffer Exchange Procedure

(See Figure 1.)

1. Select the most appropriate MWCO for your sample. For maximum recovery, select a MWCO ½ to 2 the molecular size of the species of interest.
2. Fill concentrator with up to the maximum volume stated in the device operating instructions*, (e.g. 20 ml if Vivaspin® 20 is used).
3. If the sample is smaller than the maximum device volume*, it can be diluted up to the maximum volume before the first centrifugation step. This will help increase the salt removal rate.
4. Centrifuge for the recommended amount of time at an appropriate spin speed for your Vivaspin® model*.
5. Empty filtrate container†.
6. Refill concentrator with an appropriate solvent.
7. Centrifuge again as before.
8. Empty filtrate container†.
9. Recover the concentrated, de-salted sample from the bottom of the concentrate pocket with a pipette.

Notes

* For guidance on maximum fill volumes, spin speeds and suggested spin times, please refer to the Operating Instructions that accompany your Vivaspin® products.

† Filtrate volumes should be retained until the concentrated sample has been analyzed.

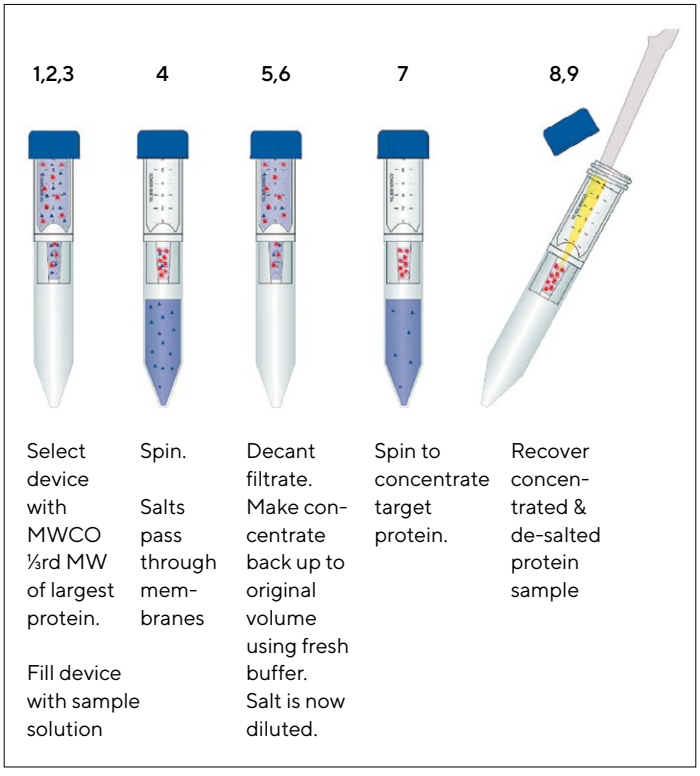

Figure 1: Step-by-step method for desalting and concentration

Test Results

As the results below show, the efficient design of Vivaspin® devices allowed >95% of the salt to be removed during the first centrifugation step. Only one subsequent centrifugation step was needed to increase the typical salt removal to 99% with >92% recovery of the sample.

Vivaspin® 20

MWCO	5 kDa		30 kDa		50 kDa		100 kDa	
	Cytochrome C 0.25 mg/ml		BSA 1 mg/ml		BSA 1 mg/ml		IgG 1 mg/ml	
	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal
Spin 1	100%	99%	97%	99%	97%	99%	90%	98%
Spin 2	96%	100%	92%	100%	93%	100%	87%	100%

Four Vivaspin® 20 devices of each cut-off were tested with 20 ml of solution. Each of the solutions contained 500 mM NaCl. Each spin was performed at 4,000 × g.
The devices > 5kDa were spun for 30 min. The devices with 5 kDa were spun 45 min. After the first and second spin, the retentate was brought up to 20 ml with ultra pure water from the Arium system (Sartorius). OD readings were taken at 410 nm for the Cytochrome C and 280 nm for the BSA and IgG samples. Salt concentration was measured with a Qcond 2200 conductivity measuring instrument.

Vivaspin® 6

MWCO	5 kDa		30 kDa		50 kDa		100 kDa	
	Cytochrome C 0.25 mg/ml		BSA 1 mg/ml		BSA 1 mg/ml		IgG 1 mg/ml	
	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal
Spin 1	98%	99%	92%	99%	93%	99%	92%	98%
Spin 2	85%	100%	86%	100%	83%	100%	89%	100%

Four Vivaspin® 6 devices of each cut- off were tested with 6 ml of solution. Each of the solutions contained 500 mM NaCl. Each spin was performed at 4,000 × g.
The devices > 5 kDa were spun for 30 min. The devices with 5 kDa were spun 45 min. After the first and the second spin the retentate was brought up to 6 ml with ultra pure water from the Arium system (Sartorius) OD readings were taken at 410 nm for the Cytochrome C and 280 nm for the BSA and IgG samples. Salt concentration was measured with a Qcond 2200 conductivity measuring instrument.

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Application Note

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Keywords or phrases:
Concentration ratio, final volume adjustment

Concentration to a Defined Final Volume With Vivaspin® Turbo 15, Vivaspin® Turbo 4 and Vivaspin® 500

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Abstract

This short Application Note describes how you can use Vivaspin® Turbo 15, Vivaspin® Turbo 4 and Vivaspin® 500 concentrators to concentrate to defined final volumes. By adding a particular volume to the filtrate vessel prior to the concentration, the final volume of the concentrate can be adjusted accurately.

Introduction

It is sometimes desirable to be able to preselect a defined final volume for a concentration step, especially when parallel concentrations are being performed. Vivaspin® centrifugal concentrators have a built-in deadstop feature, which prevents overconcentration to dryness. Due to the fast concentration rates possible with the patented vertical membrane design in the Vivaspin®, the drying out of the sample would otherwise be a possibility.

This note describes a method for achieving reproducible defined final volumes using Vivaspin® Turbo 15, Vivaspin® Turbo 4 and Vivaspin® 500 centrifugal concentrators. The method does not rely on the deadstop pocket but is increasing the retained volume by adding liquid to the filtrate vessel prior to centrifugation.

Equipment

- Vivaspin® Turbo 15 10kDa MWCO
- Vivaspin® Turbo 4 10kDa MWCO
- Vivaspin® 500 10kDa MWCO
- Tacta 5 ml mechanical pipette and Optifit pipette tips
- Tacta 1000 µl mechanical pipette and Optifit pipette tips
- Tacta 200 µl mechanical pipette and Optifit pipette tips
- Arium® pro ultrapure water system
- Sartorius Precision Lab Balance
- Centrisart® D-16C Centrifuge with swing out rotor for 50 ml and 15 ml falcon tubes
- Centrisart A-14C Centrifuge with fixed angle rotor for 24 1.5 | 2.2 ml tubes

Reagents

1 mg/ml Bovine Serum Albumin labelled with Bromophenol blue

Methods

1. Add defined amount of water to the filtrate tube (see table next page).
2. Put the concentrator insert into the filtrate tube and add sample solution.
3. Close the concentrator screw cap (for Vivaspin® Turbo 15 or Vivaspin® Turbo 4) or close the cap (Vivaspin® 500) and place in the centrifuge.
4. Concentrate the sample.
5. Remove the concentrator insert and recover the concentrate with a pipette.

Results

Results for Vivaspin® Turbo 15

Volume of water added to the filtrate tube	Volume of sample solution added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
11.5 ml	15 ml	20 min @ 4,000 × g	1.50 ± 0.02 ml
9.5 ml	15 ml	20 min @ 4,000 × g	0.96 ± 0.01 ml
7.5 ml	15 ml	20 min @ 4,000 × g	0.53 ± 0.02 ml

Results for Vivaspin® Turbo 4

Volume of water added to the filtrate tube	Volume of sample solution added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
2.0 ml	4 ml	20 min @ 4,000 × g	0.34 ± 0.03 ml
1.5 ml	4 ml	20 min @ 4,000 × g	0.15 ± 0.02 ml
1.2 ml	4 ml	20 min @ 4,000 × g	80 ± 10 µl

Results for Vivaspin® 500 in 40° fixed angle rotor

Volume of water added to the filtrate tube	Volume of sample solution added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
500 µl	500 µl	15 min @ 15,000 × g	103 µl ± 13 µl
380 µl	500 µl	15 min @ 15,000 × g	51 µl ± 11 µl
250 µl	500 µl	15 min @ 15,000 × g	30 µl ± 5 µl
200 µl	500 µl	15 min @ 15,000 × g	23 µl ± 7 µl

Conclusion

Reproducible defined final concentrate volumes can be quickly and easily achieved with Vivaspin® Turbo 15, Vivaspin® Turbo 4, and Vivaspin® 500.

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Passivation, non-specific binding, protein concentration, low volume

Treatment of Vivaspin® Concentrators for Improved Recovery of Low-Concentrated Protein Samples

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Abstract

This application note shows how to significantly reduce the loss of low-volume protein sample. The non-specific binding properties of the Vivaspin centrifugal ultrafiltration device membranes and housing materials allows for protein-saving protocols. Any loss of sample in the nano – microgram range may be negated through a device "passivation" procedure, whereby pre-treatment of the concentrators with certain blocking solutions will reduce the available non-specific ligands that may otherwise bind to the target protein.

Introduction

With appropriate device size and membrane cut-off selected, Vivaspin® products will typically yield recoveries for the concentrat- ed sample > 90% when the starting sample contains over 0.1 mg/ml protein of interest. Depending on sample characteristics rela- tive to the membrane type used, solute (protein) adsorption on the membrane surface is typically very low (2 - -10 µg/cm^2) and in practice not detectable.

This can increase to 20 – 100 µg/cm^2 when the filtrate is of interest and the sample must pass through the whole internal structure of the membrane. Whilst the relative adsorption to the plastic of the sample container will be proportionately less important than on the membrane, due to the higher total surface area, this can be also be a source of yield loss. Typically, a higher cut-off membrane will bind more than a low molecular weight alternative.

Whenever possible, the smallest MWCO and device size applicable should be chosen. Swinging bucket rotors are preferred to fixed angle rotors. This reduces the surface area of the concentrator that will be exposed to the solution during centrifugation.

An important factor not to be neglected is the thorough recovery of the retentate. Make sure to carefully remove all traces of solution from the sample container and, if feasible, rinse the device after recovering the sample with one or more drops of buffer and then recover again.

The intention of the following "passivation" procedure is to improve recovery of protein samples in the nano- to microgram concentration range by pretreating the device (membrane & plastic). For this purpose a range of solutions are suggested in Table 1.

Type	Concentration
Powdered milk	1% in Arium water
BSA	1% in PBS
Tween 20	5% in Arium water
SDS	5% in Arium water
Triton X-100	5% in Arium water
PEG 3000	5% in Arium water

Table 1: Passivation Solutions

Methods

Passivation procedure for Vivaspin® ultrafiltration concentrators

A) Passivation Procedure

1. Wash the concentrators once by filling with Arium water and spin the liquid through according to the respective protocol.
2. Remove residual water thoroughly by pipetting. Caution: Take care not to damage the membrane with the pipette tip.
3. Fill concentrators with the blocking solution of choice as given in Table 1.
4. Incubate the filled concentrators at room temperature for at least 2 hours (overnight is also possible except for Triton X-100 which is not recommended for overnight incubation).
5. Pour out the blocking solution.
6. Rinse the device 3 – 4 × very thoroughly with Arium water and finally spin through.
7. The "passivated" devices are now ready for use. We recommend comparing different passivation reagents with an untreated device.

Note

It is necessary to rinse the device thoroughly before each washspin to ensure that traces of passivation compound are removed from the deadstop. Use the device immediately for protein concentration or store it at 4°C filled with Arium water, to prevent the membrane from drying.

B) Evaluation of passivation effects (exemplary with BSA)

1. Prepare a 10 µg/ml BSA stock solution e.g. by diluting 90 µl of the 4 mg/ml stock solution in 36 ml 0.1 M sodium borate pH 9.3. Mix well.
2. Fill Vivaspin® 2 devices with 2 ml of this 10 µg/ml BSA solution and close with cap provided.
3. Spin the device in a swing-out rotor at 4,000 + g until the volume is to app. 100 µl.
4. Recover the concentrate and make back up to 2 ml with 0.1 M sodium borate pH 9.3
5. Determine recovered protein concentrations e.g. according to Bradford or BCA assays.

Results and Discussion

As an example, the effect of milk powder was analysed. It could be shown (Figure 2) that the protein recovery of a 10 µg/ml BSA solution could be increased from around 70 to 90%. If milk powder is not interfering with sample purity and quality, it is a good starting point to improve recovery of diluted sample solutions.

Protein recovery (10 µg/ml BSA) with Vivaspin® PES 10 kDa after passivation

In another example, detergents were analysed with only 250 and 500 ng BSA (Figure 3). BSA recovery declined to 50 - 30% in untreated devices as the protein concentration was reduced. Significant improvement to 60 - 90% recovery could be demonstrated when using the passivation strategy. Often, Triton X-100 seemed to work though the optimal reagent has to be selected for the respective protein and its hydrophilic | -phobic characteristics.

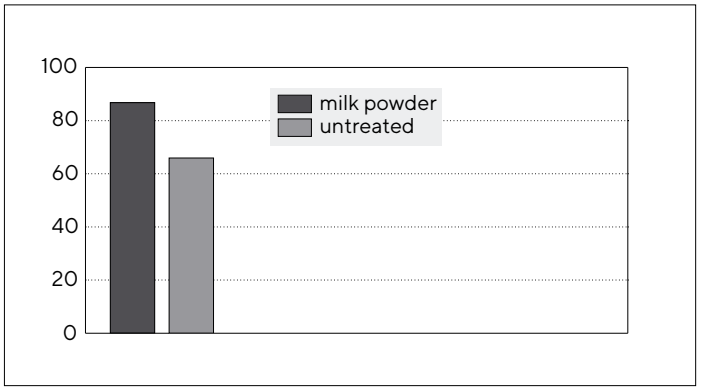

Figure 2: Protein recovery (10 µg/ml BSA) with Vivaspin® PES 10 kDa after passivation

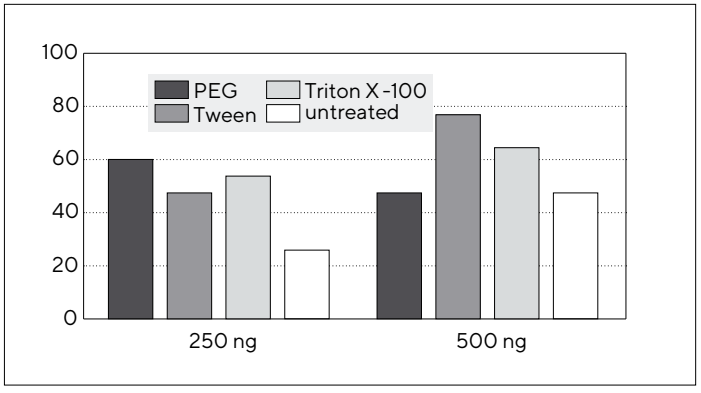

Figure 3: Protein recovery (250 and 500 ng BSA) with Vivaspin® 2 PES 10 kDa after passivation

Summary

Passivation is an appropriate method to achieve increasing sample recovery when using very dilute samples. In addition to skimmed milk, other proteins (BSA), detergents and compounds are possible. However, it should be noted that this is a general procedure, not specific for any particular application. Depending on the hydrophilic | -phobic character of the protein non-specific binding may be more or less of a problem and the suggested passivation solutions may lead to different results. Even with the Hydrosart membrane, which is recommended for dilute samples, passivation of the device will reduce losses on the plastic surface. One very important thing to remember is that the blocking agent is potentially introduced into the sample. It should be assured that this will not interfere with downstream analysis. For example, proteins must not be used for passivation if a pure protein is intended to be concentrated for x-ray crystallography, as even the smallest traces would interfere with the diffraction pattern. Other subsequent analyses methods include activity testing, gel electrophoresis or labelling are less problematic.

Additional application notes like those highlighted below, plus many more, can be found on:
www.sartorius.com/labultrafiltration

- Sartorius Ultrafiltration Products in the Preparation of Biological Nanoparticles and Medical Nanocarriers – a Short Review
- Vivaspin® 500 High-cell-density Cultivation of Ultrafiltration: a Substitute to Lyophilization
- Concentration of Mammalian Cell Culture Supernatants with Vivaspin® Turbo 15
- Vivaflow® 200: A Critical Sample Preparation Tool for Concentration Hybridoma Supernatants
- Scouting Protein Purification Conditions Using Vivapure® Centrifugal Ion Exchange Membrane Absorbers
- Removal of Endotoxin from Monoclonal Antibodies
- Vivaflow® and Vivaspin® Workflow in Protein Research Laboratories
- Vivaspin® 20 with Sartorius Diafiltration Cups: Comparing Their Performance in Buffer Exchange to Conventional Dialysis Cassettes
- High Recovery of Cationised Protein in Centrisart® I
- Urine Protein Concentration with Vivaspin® Concentrators
- Vivaspin® Turbo 4 Ultrafiltration – An Economic and Ergonomic Approach to Separation Protein & Metabolites for Disease Detection
- Primer Removal After a PCR Reaction with Vivacon® 2

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